Oral Presentations

Oral Abstracts

1. **LUBRICIN AND HOW ITS CARBOHYDRATE-PROTEIN AND PROTEIN-PROTEIN INTERACTIONS PROVIDE ITS FUNCTIONALITY**
   
   **Purpose**: Biolubrication is key for sustaining the mobility of joints. The consequence of faulty biolubrication is pronounced in pathological conditions such as osteoarthritis (OA) and rheumatoid arthritis (RA), where degradation of the joint is exacerbated by defect of the lubricating superficial layer on the cartilage. For OA and RA, molecular investigation of this layer and its molecules in healthy and disease conditions is crucial.

   **Methods**: We have characterised lubricin in the synovial fluid using proteomic and glycomic techniques. Lubricin was found to be associated with extracellular matrix (ECM) proteins of joint tissue and this association was verified in vitro using recombinant protein constructs of lubricin and its identified binding partners. Immunohistological staining was also used to identify the specific staining of lubricin to cartilage (protein interaction) and to synovial neutrophils (carbohydrate interaction).

   **Results**: Our data suggest that part of lubricin becomes linked to the Cartilage Oligomeric Matrix Protein (COMP) via covalent and non-covalent interaction. This association to a cartilage located protein explains how lubricin can provide efficient boundary lubrication even under high stress conditions in a healthy joint by its specific interaction to cartilage. This organization allows the glycosylated mucin domain of lubricin to generate a friction free joint surface. Analysis of oligosaccharides from OA patients suggests that there are pathological changes that could influence the lubrication property. Also, identification of complex oligosaccharides present in healthy and diseased state indicates that its glycosylation may have additional function as an immune regulator.

   **Conclusions**: The data suggest that the mechanisms for localization of the surface active lubricin to synovial surfaces provide insight into transformation from a healthy state to pathological state, including also changes of the glycosylation that would directly link to the pathology found in OA and RA.

2. **LUBRICATION OF CARTILAGE — MENISCUS BIOINTERFACE BY PROTEOGLYCAN 4 AND HYALURONAN; EFFECT OF SLIDING VELOCITY**

   **Purpose**: The meniscus is a fibrocartilaginous pad in the knee joint that has load bearing and lubricating properties. The meniscus provide these properties during joint loading through articulating against the cartilage of the femur superiorly and the tibia inferiorly. Removal or damage of the meniscus can lead to changes in joint loading and sub-chondral bone structure, as well as higher prevalence of chronic bone degradation, or osteoarthritis (OA). Meniscal lesions are prevalent in the middle-aged and elderly (ranging 19% of women aged 50-59 to 56% of men aged 70-90); similarly 50% of people aged over 65 suffer from OA. The meniscus forms a biointerface with articular cartilage within the synovial joint. Friction between the apposing surfaces is modulated through several distinct modes of lubrication. Boundary mode lubrication occurs during surface-to-surface contact where surface bound molecules effect lubrication, generally in high load and low speed conditions.

   Proteoglycan 4 (PRG4) and hyaluronan (HA) are lubricating molecules present within synovial fluid (SF). PRG4, or lubricin, is a lubricating glycoprotein that contributes to, and is necessary for, proper joint function. PRG4 is synthesized and secreted by cells near the surface of cartilage, as well as the meniscus, and is present at the articulating surfaces of these tissues. Both PRG4 and HA, a repeating disaccharide, have been shown to be effective, dose-dependent boundary lubricants at an articular cartilage-cartilage biointerface in vitro. HA and PRG4 also interact synergistically to lower friction to levels similar to that of whole SF. However, the lubricating properties of these two lubricants at an articular cartilage-meniscus biointerface are currently unknown. Therefore, the objective of this study was to characterize the boundary lubricating properties of PRG4 and HA, both alone and in combination, using an in vitro cartilage-meniscus boundary lubrication friction test.

   **Methods**: A custom cartilage-on-cartilage friction test setup was modified to articulate the surface of meniscus tissue against that of articular cartilage. Tissue samples were harvested from fresh mature bovine stifles joints. Osteochondral annuli were harvested from the patellofemoral groove. Meniscal tissues, with the articular surface in tact, were fashioned into ~12mm diameter disks from the inferior surface. The effect of sliding velocity on the friction coefficients at this articular cartilage-meniscus biointerface was evaluated to identify conditions where a boundary mode of lubrication is operative. PRG4 at 0.450 mg/ml, 1500 kDa HA at 3.33 mg/ml, both alone and in combination, were the lubricants of interest. Phosphate-buffered saline (PBS) and SF served as negative and positive controls, respectively. Static friction, $\mu$ static, Neq, and kinetic friction, $\mu_k$ Neq, coefficients were calculated. Data is presented as mean±SEM, N=5–17.

   **Results**: PRG4 and HA demonstrated lubricating function, both alone and in combination. PBS had highest values of $\mu_k$ Neq at all sliding velocities, while SF had the lowest. PRG4 lowered friction at low sliding velocities ($0.01$–$1$ mm/s), while both HA and PRG4–HA lowered friction approaching the level of SF (Fig. 1). At a sliding velocity of 0.03 mm/s, PBS had a $\mu_k$ kinetic, Neq of 0.145±0.015, which decreased to 0.105±0.018 in PRG4 and 0.083±0.009 in HA. PRG4–HA was 0.070±0.010, approaching 0.046±0.003 of SF. Similar trends were observed for $\mu$ static, Neq.